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Outbreak of *Burkholderia cepacia* Bacteremia in a Pediatric Hospital Due to Contamination of Lipid Emulsion Stoppers

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We describe a 7-month outbreak of nosocomial *Burkholderia cepacia* bacteremia involving eight children in a pediatric hospital and the results of epidemiological investigations. A *B. cepacia* strain genotypically identical to the blood isolates was recovered from the upper surface of capped rubber stoppers of bottles of a commercial lipid emulsion used for parenteral nutrition.

Burkholderia (previously *Pseudomonas*) *cepacia* is a gram-negative bacillus commonly found in soil and moist environments and capable of surviving and growing in nutrient-poor water (7). *B. cepacia* often colonizes the lungs of patients with cystic fibrosis (10) and has emerged as an important opportunistic pathogen in hospitalized and immunocompromised patients (1, 7, 12, 19). Small hospital outbreaks are frequent and are usually due to a single contaminated source such as disinfectant (16), intravenous solutions (19), nebulizer solutions (9), mouthwash (5), and medical devices, including respiratory-therapy equipment (13, 20). *B. cepacia* pseudobacteremia is mentioned in several reports (2, 8, 16), but true bacteremia is rare.

We describe a nosocomial outbreak of *B. cepacia* bacteremia occurring between October 2001 and April 2002 in three wards of our pediatric hospital and report the results of epidemiological investigations.

The first cases occurred in a 31-bed neonatal intensive care unit (NICU). From 26 to 28 October 2001, *B. cepacia* bacteremia was diagnosed in four premature newborns aged from 5 to 33 days. The first case involved a neonate (gestational age, 33 weeks; birth weight, 1,290 g) whose umbilical catheter was removed on day 5 of life (26 October); a culture of the distal segment grew 10^6 CFU of *B. cepacia*/ml by Brun-Buisson's method (4) (isolate 1). Blood grown in cultures (FAN Aerobie; Biomérieux, Marcy l'Etoile, France) on day 6 of life grew *B. cepacia*, and the infant was successfully treated with ceftazidime (100 mg/kg of body weight/day for 10 days). During the following 2 days, three other premature newborns (26 weeks, 870 g; 30 weeks, 1,000 g; and 31 weeks, 1,700 g) showed clinical deterioration, and qualitative and quantitative blood cultures grew *B. cepacia* (isolates 2, 3, and 4). The central percutaneous catheters were removed, and all three infants recovered on antibiotic therapy chosen according to in vitro susceptibility (ceftazidime at 200 mg/kg/day plus ciprofloxacin at 30 mg/kg/day for 15 days).

At 3 weeks later, in the 18-bed gastroenterology intensive care unit of our institution a 16-month-old child with multiple congenital malformations and Hirschsprung syndrome requiring parenteral nutrition developed *B. cepacia* bacteremia (isolate 5). The feeding line could not be removed, and bacteremia recurred 2 days after completion of initial antibiotic therapy (ceftazidime at 200 mg/kg/day plus ciprofloxacin at 30 mg/kg/day for 3 weeks). The same antibiotic treatment was again instituted, and the infant recovered after Broviac catheter removal.

On 17 April 2002 in the 22-bed pediatric intensive care unit (PICU) of our institution, two premature neonates (28 weeks, 1,060 g, and 26 weeks, 750 g) in the same room developed *B. cepacia* bacteremia (isolates 6 and 7). Both infants recovered on antibiotic treatment (ceftazidime at 200 mg/kg/day plus ciprofloxacin at 30 mg/kg/day for 15 days) without central percutaneous catheter removal.

All seven patients had biological signs of sepsis (reduced platelet count and elevated C-reactive protein level), and six patients had clinical signs of septic shock (fever > 38°C, hypothermia < 37°C, and/or bradycardia and/or apnea).

Environmental and epidemiological investigations were conducted to identify the source and route of infection. Environmental samples ($n = 105$) covered sources previously reported to be potential reservoirs of *B. cepacia* and included the water reservoir of incubator humidifiers, heated humidifier water from respiratory devices, tap water, sink drains, incubator surfaces, and antiseptic products. Water samples of 100 ml were filtered (0.45-μm-pore-size filter; Millipore, Molsheim, France). Filters and surface swabs were inoculated on specific agar (*Pseudomonas cepacia* gelose; AES Laboratoire, Combourg, France) and incubated at 37°C for 72 h. Antiseptic products (alcoholic chlorhexidine and povidone-iodine) and parenteral nutrition solutions were inoculated in brain heart infusion broth (Bio-Rad, Marnes-la-Coquette, France) and incubated at 37°C for 5 days. Samples of the intravenous solution administered to all the patients were collected from the stocks of each ward and from the central

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pharmacy (one sample of each different lot). They were inoculated in blood culture bottles (FAN Aerobie; Biomérieux), which were placed in an automate apparatus for 6 days (BacT/Alert system; Biomérieux). *B. cepacia* isolates were identified on the basis of colony morphology and biochemical characteristics (NE system; API, La Balme les Grottes, France).

In the three wards, tracheal and fecal samplings were done routinely to determine colonization status and to adapt antibiotic therapy. Samples were obtained at admission and then weekly. For each patient with *B. cepacia* bacteremia, moreover, new samplings were performed after the first positive-testing blood culture to investigate the route of infection. Infection-control professionals reviewed the medical, surgical, and treatment history of all the patients with positive-testing *B. cepacia* blood cultures.

Ribotyping (after digestion by EcoRI as previously described) (3) was used to investigate the relatedness of the clinical and environmental *B. cepacia* isolates. Epidemiological isolates were compared to 46 genetically unrelated strains recovered throughout France from sputum of cystic fibrosis patients (collection of the French Observatoire Cepacia/Vaincre la Mucoviscidose). Cluster analysis (unweighted pair group method with arithmetic mean) performed on a computer running whole-band analyzer software (Biogène; Vilber-Lourmat, Marne la Vallée, France) was used to calculate similarity-dissimilarity.

None of the environmental samples or antiseptic products grew *B. cepacia*. All the patients had a central venous catheter (umbilical, central percutaneous, or Broviac) for parenteral nutrition, and one patient was mechanically ventilated. In five of the seven cases, quantitative blood culture or quantitative catheter culture incriminated the catheter as the source of bacteremia. None of the patients had tracheal or digestive colonization before or after the onset of bacteremia. During the first and third episodes, the cases clustered in a very short period (<48 h), pointing to direct access of the pathogen to the bloodstream and to the existence of a common and transient exogenous source. Parenteral nutrition solutions were prepared in the pharmacy for six patients and in the NICU for one patient. Samples of each patient's parenteral nutrition solution stored in the pharmacy were sterile.

During the first and second episodes, epidemiological investigations showed that the only intravenous solution shared by all the children was a lipid emulsion (Ivelip; Baxter/Clintec Parenteral SA, Montargis, France) (20%; 100 ml), and subsequent investigations focused on this product. This lipid emulsion was frequently used (for about 75% of patients) in both wards, and the same bottle was sometimes used for several patients in the NICU. Ten bottles of this product (randomly chosen during the two episodes) were all culture negative. At this time, the source of contamination was unknown.

During the third episode, Ivelip was again the only factor in common with the preceding episodes. Thus, 50 bottles of Ivelip were chosen randomly for culture. After removing the plastic flip-off caps covering the rubber stoppers, an investigator noted a drop of condensation on top of the stoppers of 25 bottles. Culture of these drops yielded about 10^5 CFU of *B. cepacia*/ml in two cases (isolate 8).

The manufacturer was immediately informed, and additional investigations were carried out. A total of 50 bottles stored in the manufacturing plant after autoclaving were examined, and *B. cepacia* was found on the stopper of one of them (isolate 9). *B. cepacia* was subsequently found in the autoclave cooling water and also in residual water remaining inside the autoclave after a sterilization cycle.

After the third outbreak, a retrospective investigation was launched in all Paris hospitals (Assistance Publique, Hôpitaux de Paris). Four other cases of *B. cepacia* bacteremia were identified between September 2001 and April 2002. Only one of these patients, a 7-month-old girl, had received Ivelip infusion (blood isolate 10). A national alert was issued, and the batch concerned was recalled. Production was halted in the manufacturing facility until the problem was solved.

The manufacturer cooperated closely throughout the investigations.

The isolate from the autoclave cooling system was lost before it could be genotyped. All eight clinical isolates and all the isolates recovered from the stoppers of bottles stored in the hospital and in the manufacturing plant shared the same ribotype, which differed from the ribotypes of the 46 unrelated control strains (Fig. 1). This confirmed that the eight cases of bacteremia were due to external contamination of the rubber in the manufacturing plant.

Several perfusion-related outbreaks of bloodstream infection have been reported, usually involving staphylococci or *Candida* spp. (18). Gram-negative bacilli such as *Enterobacter*, *Pseudomonas*, *Citrobacter*, and *Serratia* spp. have also been incriminated (6, 14, 15). Parenteral solutions and lipid emulsions, which can support the growth of many bacteria and fungi, are frequently involved (6, 11). However, the overall contribution of contaminated perfusates to catheter-related bacteremia remains very small (18).

Manufacturers guarantee the sterility of the solution itself but not that of the outside of the container, including the stopper. Moreover, flip-off caps are not designed to be hermetic, and the presence of a drop of condensation water on the cap is not uncommon and is inherent to the design of the flip-off cap (internal correspondence with manufacturer, April 2003). A nationwide epidemic of *Enterobacter* septicemia occurred in the United States in 1970 to 1971 due to contamination of the inner surface of screw-cap closures of various infusion bottles after autoclaving in the manufacturing plants (14). The source of contamination was not clearly established. Solutions for infusion can also be contaminated during manipulation (11, 17, 19) or by the use of contaminated antiseptic products for cap decontamination (2).

In the outbreak we describe, viable bacteria gained access to the top of the stopper, probably during cooling after autoclaving. The water used to cool the product externally during the last step of the cycle was water sterilized during the product sterilization cycle. In our case, a leak in an autoclave valve has been detected which led to contamination of the water used for the cooling step. *B. cepacia* persisted in this nutrient-poor environment and was inoculated into the intravenous fluid when the stopper was perforated before use. An inquiry revealed inadequate disinfection of the caps and stoppers and especially an inadequate antiseptic contact time. Cap disinfection was done by swabbing with alcoholic chlorhexidine in PICU and NICU and with povidone-iodine in gastroenterology

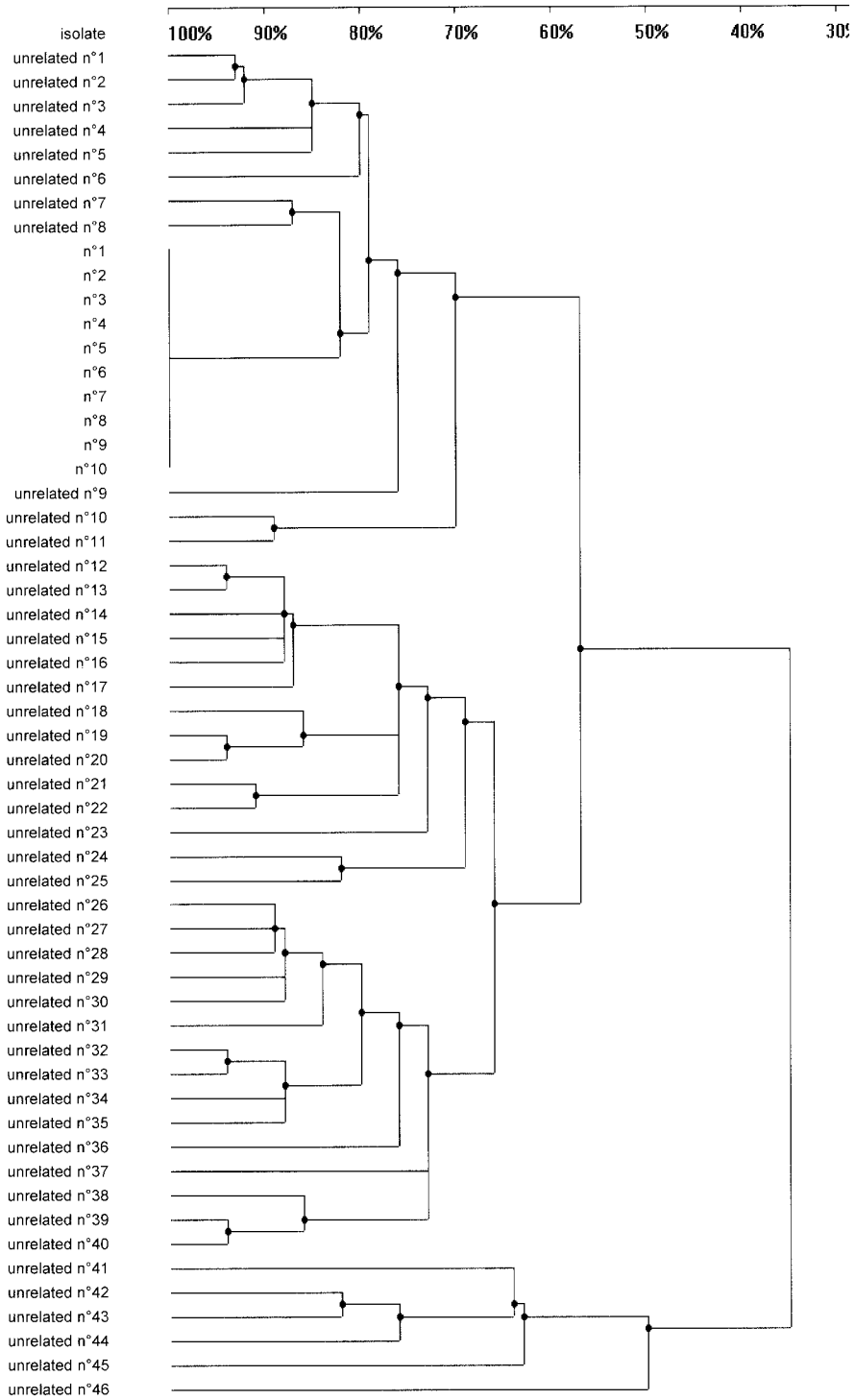

FIG. 1. Dendrogram based on the ribotypes of the *B. cepacia* isolates: clinical isolates recovered during the outbreak (n°1 to 7 and 10), isolates recovered from the stoppers of lipid emulsion bottles (n°8 and 9), and unrelated isolates from the collection of the French Observatoire Cepacia/Vaincre la Mucoviscidose (n°1 to 46).

intensive care units. Generally, the (insufficient) contact time was <10 s. The outbreaks in the NICU and PICU were due to the use of a single bottle for several patients. Because small volumes of emulsion are required for neonates, individually assigned disposable syringes were prepared from the same bottles during the same session under laminar airflow protection. Thus, the outbreaks were probably due to several dysfunctions, including a defective autoclaving process, inadequate cap disinfection, and multiple use of a given bottle of perfusate. Contributory factors included the long infusion pe-

riod at room temperature (18 to 20 h) and the vulnerability of the patients (six of the seven infants were premature and had low birth weights). Corrective actions taken were proscribing the multiple use of the same bottle and giving written instructions on the correct disinfection procedure to the nurses.

Several lessons can be drawn from this outbreak. (i) Contaminated intravenous fluids are a rare source of catheter-related bacteremia, but this possibility should be borne in mind, especially when bacteremia is due to an environmental microorganism. (ii) Epidemiological investigations are required to identify the source and route of infection. In the outbreak we describe, the source was identified by clinical investigations. Administration of the lipid emulsion was the only feature shared by all the patients, but it was also a very frequent treatment in the wards concerned, and most of the patients thus treated did not develop *B. cepacia* bacteremia. This source was confirmed despite initially negative results in the testing of microbiological controls. (iii) The risk of post-autoclaving contamination must not be underestimated. (iv) Stopper disinfection procedures prior to the use of intravenous fluid bottles must be regularly controlled, and each bottle should be used to treat a single patient.

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